Remarks

Claims 48-66 are pending in the subject application. By this Amendment, Applicants have canceled claims 53 and 66, amended claims 48, 54 and 63 and added new claims 67-70. Support for the amendments and new claims can be found throughout the subject specification and in the claims as originally filed (see, for example, the original claims; page 4, lines 19-26; page 8, lines 10-23; page 12; and page 13, lines 20-30 of the as-filed description). Entry and consideration of the amendments and new claims presented herein is respectfully requested. Accordingly, claims 48-52, 54-65 and 67-70 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

Applicants gratefully acknowledge the Examiner's withdrawal of the rejection under 35 U.S.C. 102(b) (over Haldimann *et al.*).

Claim 66 is objected to under 37 C.F.R. § 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. By this Amendment, Applicants have canceled claim 66, rendering this objection moot. Accordingly, reconsideration and withdrawal of the objection to the claims is respectfully requested.

Claims 53 and 66 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite. The Office Action indicates that the phrase "the origin of transfer is functional in E. coli host cells" is unclear. Applicants respectfully assert that the claims as filed are definite. Applicants have amended claim 53 in a manner that clarifies that the origin of transfer is functional in *E. coli*. Applicants note that the environmental DNA samples are propagated in *E. coli* (donor cells) within cloning vectors and then transferred into non-*E. coli* host cells (recipient cells) by conjugation. The origin of transfer must be functional in *E. coli* in order for the transfer to occur. The Office Action states that the recitation of "said environmental DNA fragments are cloned in *E. coli*" in claim 66 is confusing. As noted above, Applicants have canceled claim 66 thereby rendering this portion of the rejection moot. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 48-66 are rejected under 35 U.S.C. § 103(a) as obvious over Rondon *et al.* (2000) in view of Hoang *et al.* (2000), Chain *et al.* (2000), Groth *et al.* (2000), Berg *et al.* (1982), and if necessary in view of Devine *et al.* (U.S. Patent No. 5,728,551) and Martinez *et al.* (U.S. published

Application No. 2003/0143745). The Office Action indicates that Rondon *et al.* teach molecular cloning of DNA isolated from microbial samples using various cloning vectors. The Office Action notes that Hoang *et al.* teach methods of using vectors containing OriT and integrase to integrate desired DNA into genome of host cells and teach using plasmids having OriT and an integrase encoding gene. The Office Action states that Chain *et al.* teach inserting oriT from RK2 into cloning vectors for site specific recombination of fragments of bacterial genomic DNA isolated from environment, which read on the origin of transfer. The Office Action claims that Groth *et al.* teach inserting using phage C31 integrase to carry out recombination between DNA of interest and bacterial chromosome or human DNA. Berg *et al.* is cited as teaching inserting using transposon elements for modifying DNA contracts and teaching the transposon DNA comprising inverted repeats, and marker gene such as Kan resistance gene. The Office Action contends that Devine *et al.* teach using transposons to facilitate DNA recombinant events and the advantages of "in vitro" transposon reactions such as high efficiency and versatility of the method. Finally, Martinez *et al.* is cited as teaching using various cloning and/or integrating vectors to insert DNA fragments of interest into the genome of host cells other than *E. coli* such as *Streptomyces* and specifically teach using vectors containing a cassette comprising OriT and phage C31 integrase. Applicants respectfully assert that the claimed invention is not obvious over the cited references.

On page 11 of the Office Action, it is argued that a person of ordinary skill in the art would have been motivated to insert a cassette having oriT and a gene encoding for an integrase to a vector and using the vector to integrate the DNA of interest into the genome of the desired host cell. Applicants respectfully disagree with this position. Applicants submit that a person of ordinary skill in the art, wanting to use a vector comprising an oriT and a gene encoding for an integrase to screen a DNA library, would have used a vector already comprising these two elements to construct the metagenomic library and would not have been motivated to insert these elements into vectors containing environmental DNA fragments of interest after construction of the library.

Constructing the vector with such elements prior to the insertion of a DNA fragment of interest is taught in all cited documents related to environmental DNA library screening methods, *i.e.,* Rondon *et al.*, Hoang *et al.* and Martinez *et al.* In these documents, the cloning vector is completely constructed, including the oriT and integrase elements, before insertion of the DNA

fragment of interest. After the insertion of the DNA fragment of interest, the complete vector is used to transform *E. coli*. After a first screening, the vector can be transferred into a new host cell (*E. coli* or others) for which the vector already contains every needed genetic element. Applicants submit that there is no motivation to insert a second set of oriT and integrase elements into vectors that already contain such elements.

Applicants further note that it is argued that Rondon *et al.* teach modification steps of selected vectors. In Rondon *et al.*, the modifications are: a transposon mutagenesis (page 2542, right column, "transposon mutagenesis") to sequence the DNA fragment of interest from transposon ends and to identify open reading frames encoding a characteristic or phenotype of interest by insertional inactivation via transposon mutagenesis (it has to be noted that in this case it is better if the transposon is inserted into the gene encoding a characteristic or phenotype of interest, see, for example, page 2544); and the sonication of clone SL1-36C7 (page 2542, right column, "sequencing clone SL1-36C7", paragraphs 4-5) in order to fragment the vector. The vector fragments are then cloned into a new plasmid in order to proceed to sequencing reactions. Applicants submit that either of these modifications leads to the possible inactivation of the DNA fragment of interest (for instance by transposon insertion) or to the destruction of the selected vector. Accordingly, these modifications are not suitable to modify the original cloning vector in order to change host cell or to continue the screening and, as such, fail to meet the limitations of the claimed invention.

Applicants also submit that the skilled person would not have been motivated to insert a polynucleotide (comprising an oriT and a gene encoding for an integrase) into a vector that already contains a DNA fragment of interest due to the intrinsic risk of this type of manipulation to alter the DNA fragment of interest. Applicants submit that modifying the vector after the construction of the library according to the invention is needed only to solve a problem which is absent from the cited documents; namely, the screening of the library in a ***plurality*** of different expression systems (a plurality of different host cells) to increase the probability to detect particular activities.

To perform this screening in multiple different host cells, it is necessary to maintain the library in a cell, such as *E. coli*, and to modify selected vectors to allow transfer and integration in each other selected recipient host cell. Genetic elements needed for the transfer and the integration may be different for every selected expression system (recipient host cell). When the library is

constructed to be expressed only in one or two expression systems, *e.g.*, as in Martinez *et al.* (*Streptomyces* and *Pseudomonas)*, the skilled person would have inserted all necessary genetic elements (*e.g.*, oriT, integrases, *etc.*) into the vector prior to the construction of the library by insertion of DNA fragments containing a characteristic of interest. The skilled person has thus no reason to modify the cloning vector after the insertion of the fragment of interest. Consequently, a person of ordinary skill in the art would not have been motivated to proceed according to the method of the invention and reconsideration and withdrawal of the rejection is respectfully requested.

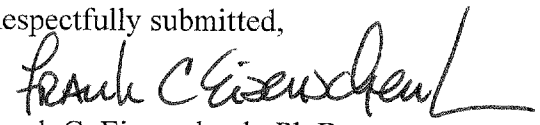
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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